GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF RESEARCH ADMINISTRATION

RESEARCH PROJECT INITIATION

Date: 22 March 1973

Project Title: "Laser Raman Studies on the Mechanisms of Cataract Lens Formation"

Project No: G-33-660

Principal Investigator: Dr. Nai Teng Yu

Sponsor: Research Corporation

Agreement Period: From March 15, 1973 Until Open

Type Agreement: Grant

Amount: $22,000

Reports Required:
Annual Reports
Final Report

Sponsor Contact Person (s):

Jack W. Powers
Regional Director
Grant Programs
Research Corporation
6075 Roswell Road N. E.
Atlanta, Georgia 30328

Assigned to: Chemistry

COPIES TO:

Principal Investigator	Library
School Director	Rich Electronic Computer Center
Dean of the College	Photographic Laboratory
Director, Research Administration	Project File
Director, Financial Affairs (2)	
Security-Reports-Property Office	
Patent Coordinator	Other

RA-3 (6-71)

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION

No action
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Date: 1/11/79

Project Title: Laser Raman Studies on the Mechanisms of Cataract Lens Formation

Project No: G-33-660

Project Director: Dr. Nai-Teng Yu

Sponsor: Research Corporation

Effective Termination Date: 11/10/78(Final Report Subm.)

Clearance of Accounting Charges: N/A - all have cleared.

Grant/Contract Closeout Actions Remaining:

_ Final Invoice and Closing Documents
_ Final Fiscal Report
_ Final Report of Inventions
_ Govt. Property Inventory & Related Certificate
_ Classified Material Certificate
_ Other _____

Assigned to: Chemistry (School/Laboratory)

COPIES TO:

Project Director
Division Chief (EES)
School/Laboratory Director
Dean/Director–EES
Accounting Office
Procurement Office
Security Coordinator (OCA) ✓
Reports Coordinator (OCA)
Library, Technical Reports Section
Office of Computing Services
Director, Physical Plant
EES Information Office
Project File (OCA)
Project Code (GTRI)
Other _____

CA–4 (3/76)

REPORT OF RESEARCH CORPORATION GRANT

(Please check one) (Submit original and one legible copy)

☒ Interim Report

☐ Terminal Report

INSTITUTION AND ADDRESS

Georgia Institute of Technology
Atlanta, Georgia 30332

PRINCIPAL INVESTIGATOR Dr. Nai-Teng Yu PHONE (404) 894-4007

ACADEMIC RANK AND DEPARTMENT Assistant Professor, Chemistry

G-33-660

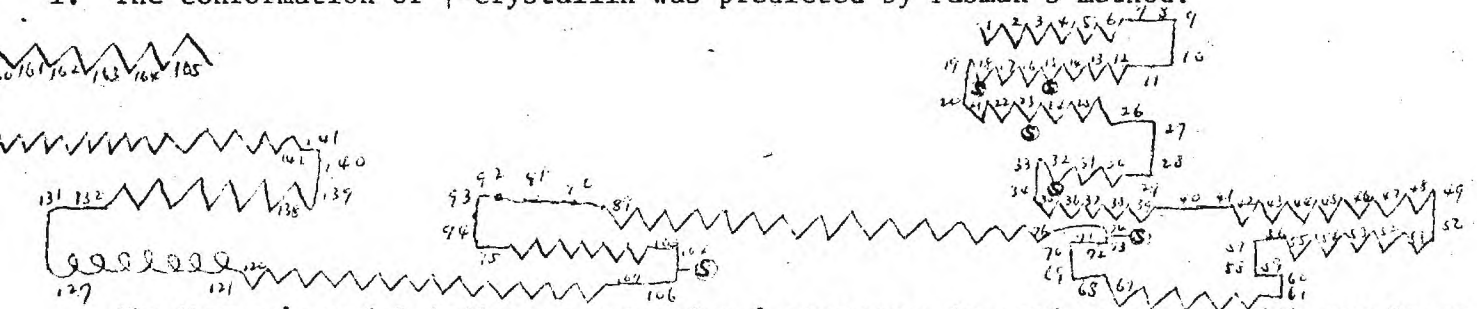
SHORT TITLE OF RESEARCH SUPPORTED BY GRANT

Laser Raman Studies on the Mechanism of Cataract Lens Formation

STARTING DATE March 15, 1973

SUMMARY OR PRINCIPAL FINDINGS AND THEIR SIGNIFICANCE (State succinctly in language understandable to one not necessarily expert in this field. Include extent to which original goals have been realized and any changes to original plan made or contemplated.)

1. The conformation of γ-crystallin was predicted by Fasman's method.



The Fasman's model indicates extensive β-structure in good agreement with our Raman results (J.B.C. 250, 2196 (1975)). One important finding in this analysis is that all six-SH groups in this protein are located near β-bends and frozen in strongly H-bonded network, incapable of being readily oxidized. Exposure of the nuclear portion of a bovine lens to 10 atm. O_2 pressure for 48 hours does not show appreciable oxidation. This suggests that the oxidation of the -SH groups are not likely to be the factor causing the formation of cataract lens. *Important findings which are not related to cataract problem but resulted from the use of our new Raman equipment purchased through this grant:

2. An empirical correlation was found between the position of the anomalously polarized line at ~1590 cm^{-1} and the distance from the center of the porphyrin ring to the pyrrole nitrogen. This relation suggests that assessment of the out-of-plane excursion of the metal is possible if the metal-to-pyrrole nitrogen distance can be estimated. The application of this method to deoxyhemoglobin yields a value of 0.39 Å as the out-of-plane displacement of high-spin Fe(II), in good agreement with the 0.42 Å found in the model high-spin iron (II) porphyrin and well below the estimated 0.70-0.83 Å by Perutz.

STUDENT PARTICIPATION (Give names of students working on the project, their role in the research, their achievements and their career plans.)

(1) Mr. C. C. Chang, Graduate Student
Role in research: Monkey Lens fractionation and Raman studies.
Achievements : (1) Isolated lens fractions: α-, β_1-, β_2-, β_3-, and γ-crystallins from monkey lens.
(2) Obtain detailed Raman spectra of these proteins.

(2) Dr. Emily J. East, Ph,D. postdoctor
Role in research: Supervise Mr. Chang's biochemical purification; spectra interpretations.
Achievements : Predict the conformation of γ-crystallin by Fasman's method and obtain a good agreement with the Raman experimental results.

PAPERS AND SCIENTIFIC TALKS (Give titles and references to papers or talks resulting from the work. Attach two copies of any reprints available, if not previously forwarded.) (1) J. Amer. Chem. Soc. 96, 4664 (1974); 2. J. Amer. Chem. Soc. 96, 3675 (1974); 3. J. Biol. Chem. 250, 2196 (1975); 4. J. Biol. Chem. 250, 1782 (1975); 5. "Resonance Raman Spectra of Metallooctaethylporphyrins" A Structural Probe of Metal Displacement", J. Amer. Chem. Soc. (in press). Talks: (1) Dept. of Biochemistry, St. Louis University, (2) 4th Internation Conference on Raman Spectroscopy, Brunswick, Maine, (3) U. S.-Japan Joint Seminar, Cleveland, Ohio (Sept. 1975).

OTHER SUPPORT (List amounts and sources—including institutional—of other contributions received or expected for this work.)

NIH GM 18894-4 $30,000 1974-1975

EXPENDITURE OF RESEARCH CORPORATION GRANT FUNDS (The terminal report should be approved by an authorized officer of the institution.)

a. Equipment, supplies (Itemize major expenditures)

Expenditure for 1973-1974.....$18,057.99
March 1974-March 1975:
Equipment: $46.50
Materials and Supplies: $642.89
Expenditure for 1974-1975.....$ 689.39

b. Stipends (Academic status, rates, periods of appointment)

C. C. Chang, Research Assistant, Summer 1974 $975.00 (3 months)

Budget	$22,000.00
Expended	$19,722.38
	$ 2,277.62

c. Other expenditures (Itemize and give purpose)

None

Signature of principal investigator ..... Date April 8, 1975

Signature of authorized officer of institution (required for terminal report only) ..... Date .....

Name and position of authorized officer of institution .....

G-33-660

REPORT OF RESEARCH CORPORATION GRANT

(Please check one) (Submit original and one legible copy)

☒ Interim Report

☐ Terminal Report

INSTITUTION AND ADDRESS

Georgia Institute of Technology
Atlanta, Ga. 30332

PRINCIPAL INVESTIGATOR Nai-Teng Yu PHONE(404)894-4007

ACADEMIC RANK AND DEPARTMENT Assistant Professor, Chemistry

SHORT TITLE OF RESEARCH SUPPORTED BY GRANT

Laser Raman Studies on the Mechanism of Cataract Lens Formation

STARTING DATE March 15, 1974

SUMMARY OR PRINCIPAL FINDINGS AND THEIR SIGNIFICANCE (State succinctly in language understandable to one not necessarily expert in this field. Include extent to which original goals have been realized and any changes to original plan made or contemplated.)

1. A reliable and simple chromatographic procedure has been developed by Dr. East for separating various fractions of lens protiens (α-, β-, γ-crystallin). The β-crystallin was further fractionated into three components (designated as β_1, β_2 and β_3). Laser Raman studies reveal that these purified proteins have exclusively antiparallel pleated sheat structure in their main-chain and that reactive sulfhydryl groups are highly localized in γ - crystallin. Our present attention is focused on the possible role of these sulfhydryl groups in the formation of cataract lens.

2. Raman scattering technique has been applied to probe the homogeneity of lens proteins structure in intact whole lens. The scattered light was collected from various parts of a bovine lens. It is found that the proteins also exist in antiparallel β structure throughout the entire lens. However, the central (nucleus) and outer (cortex) portions have quite different amino acid composition. The relative intensities of the lines at 624 (Phe) and 644 cm^{-1} (tyr) indicate that the nucleus part is richer in γ - crystallin when compared to cortex.

3. We are presently extending our techniques to the studies of human lens (both normal and cataractous).

* Important findings which are not related to cataract problem but resulted from the use of our new Raman equipment purchased through this grant:

4. We have examined resonance Raman spectra of a series of octaethylporphyrins (OEP) and found a correlation between the position of the metal ion relative to the mean porphyrin plane and the anomalous polarization of a line observed between 1509-1582 cm^{-1}. This is important because Raman methods can then be employed to obtain such information in hemeprotein systems. It is believed that the movement of the iron into the porphyrin plane upon the oxygenation of hemoglobin triggers the cooperativity behavior (manifested by the sigmoid O_2 - binding curves).

5. In recent Raman studies of α-lactalbumin, we brought foward two important points: (a) Backbone conformation of α-alactalbumin is similar to that of lysozyme, as had been suggested previously on the basis of sequence and other chemical data, (b) crystallization of α-lactalbmin does not produce any significant effect on the environment of the peptide backbone, as judged by the amide III region and lines attributable to tryptophan and cystine residues.

STUDENT PARTICIPATION (Give names of students working on the project, their role in the research, their achievements and their career plans.)

Name: Dr. Emily J. East, Ph.D. (1970), Emory University
Role in the research: Lens proteins fractionation and Raman measurements.
Achievements: (1) Successfully isolated ultra-pure lens proteins: α-, β_1-, β_2-, β_3-, and γ-crystallin from bovine lens.
(2) Obtain detailed Raman spectra of these lens fractions.
(3) Assist the principal investigation in spectra interpretations.

PAPERS AND SCIENTIFIC TALKS (Give titles and references to papers or talks resulting from the work. Attach two copies of any reprints available, if not previously forwarded.)

1. "Single-Crystal Raman Spectra of Native Insulin; Structures of Insulin Fibrils, Glucagon Fibrils and Intact Calf Lens", Arch. Biochem. Biophys., 160, 614 (1974). 2. "Comparison of Protein Structure in Crystals, in Lyophilized State and in Solution by Laser Raman Scattering: III α-Lactalbumin", J. Amer. Chem. Soc. (in press). 3. "The Structural Implication in Metalloporphyrins of the 1590 cm^{-1} Anomalously polarized Resonance Raman Line", J. Amer. Chem. Soc. (in press), Talks: (1) Polytechnic Institute of New York. (2) Biochemistry/Biophysics Conference (1974).

OTHER SUPPORT (List amounts and sources—including institutional—of other contributions received or expected for this work.)

1. Matching funds for Raman equipment $13,752.00 from School of Chem., Georgia Tech
2. NIH GM 18894 $24,735 1973-1974
3. Renewal grant application to NIH $111,910 (pending)

EXPENDITURE OF RESEARCH CORPORATION GRANT FUNDS (The terminal report should be approved by an authorized officer of the institution.)

a. Equipment, supplies (Itemize major expenditures)

1. S32D Vibration-isolated table for Raman equipment $1175.60
2. Spex triple-monochromator Raman system $13,752.00 (total cost $27504.00, School of Chemistry contributed $13,752.00).
3. HP-45 Calculator $395.00
4. Timer $89.82
5. Materials and Supplies $2645.57

Budget	$22,000.00
Expended	$18,057.99
Free Balance	$3,942.01

b. Stipends (Academic status, rates, periods of appointment)

None

c. Other expenditures (Itemize and give purpose)

None

gnature of principal investigator ..... Date May 16, 1974

gnature of authorized officer of institution (required for terminal report only) ..... Date .....